Structural Effects of Radiation Damage and its Potential for Phasing

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We have carried out a detailed analysis on the structural effects of radiation damage on the model protein thaumatin. The most pronounced structural changes observed were disulfide bond breakage and the associated main and side chain movements, as well as the decarboxylation of the aspartate and glutamate residues. The structural changes induced on the sulfur atoms were used successfully to solve the structure via a Radiation-damage Induced Phasing (RIP) procedure.

Radiation damage has been a curse of macromolecular crystallography from its early days. Until the 1990s all diffraction data were collected from macromolecular crystals mounted in glass or quartz capillaries and kept at ambient temperature, in the range of 4-20°C. Initially, collecting data from protein crystals using precession cameras or multi-circle diffractometers took a very long time. The introduction of the screenless rotation method considerably sped up this process by recording more reflections simultaneously. Still, only the most robust crystals were able to deliver all the necessary data from one specimen, and very often a complete data set had to be combined from data measured on several crystals. Although the advent of cryo-cooling techniques, where diffraction data is obtained from crystals cooled down to approximately 100K, substantially alleviated the problem of radiation damage, the obvious deterioration of cryo-cooled crystals at bright undulator beamlines revived interest and prompted several investigations into the effects of radiation damage.

Whereas previous structural radiation damage studies have been carried out at third-generation synchrotron sources, the effects of radiation damage on protein crystals can be also observed after prolonged exposures at second-generation bending magnet synchrotron beamlines. We have analyzed the effects of radiation damage on crystals of thaumatin, a medium-sized protein containing 207 amino acids, including eight disulfide bridges. We collected twenty high-resolution data sets that allowed us to perform a detailed investigation of the structural changes induced by x-ray irradiation.

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The structural changes induced by radiation damage can be visualized using difference Fourier maps that reveal the appearance or disappearance of electrons on and around the molecular model. The most striking features our analyses revealed are the breakage of disulfide bonds and the associated main and side chain movements, as shown in **Figure 1**. Less prominent, but still significant, structural changes

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involve the decarboxylation of side chains and water molecule movements.

As can be seen from **Figure 1**, the amount of electrons relocated over the course of the experiment is quite large. This suggests that the observed changes in the diffraction intensities are dominated by this structural change and that the disulfide bridges can be located using the isomorphous difference only. When this so-called substructure is located, standard procedures can be used to obtain an image of the three-dimensional electron density of the protein, which finally results in an atomic model of the protein under investigation. Normal phasing techniques use chemical or biochemical methods in order to generate a so-called derivative. In this case, the interaction of the x-rays with the protein produces a derivative suitable for phasing, hence the name Radiation-damage Induced Phasing (RIP). In the investigated case, the electron density estimates that result from various standard crystallographic computations are of such a high quality that they can be easily interpreted, as indicated in **Figure 2**.

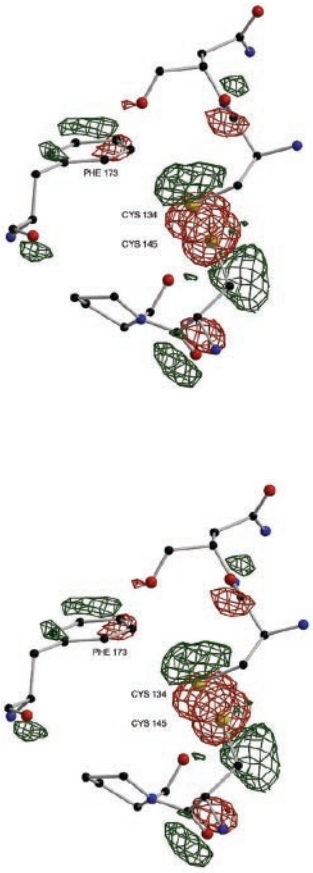

Figure 1. Positive and negative difference Fourier maps showing the disappearance of electrons in red and the appearance of electrons in green over the course of the data collection. The pattern of negative (red) and positive (green) peaks suggests that, upon the breakage of the disulfide bond CYS134-CYS145, the sulfur atoms 'swing out' while the surrounding main and side chain positions are affected.

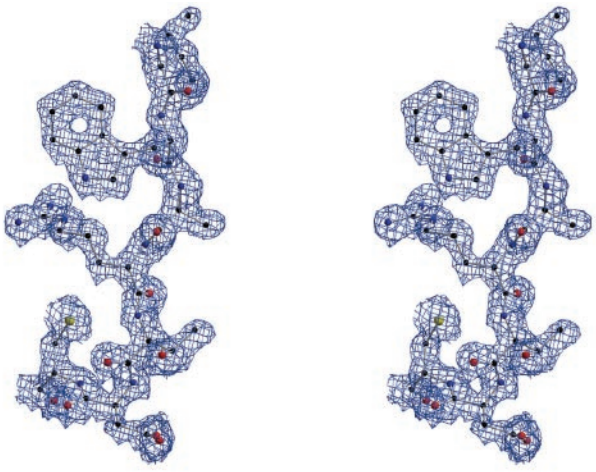

Figure 2. An electron density map calculated with solvent flattened RIP phases overlaid on the final model.